

Assays Using Human ER α and ER β

Reference	Arcaro et al. (1999)	Arcaro et al. (2000)	Fertuck et al. (2001)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER alpha and human ER beta	human ER alpha	human ER beta
<i>Source of receptor</i>	n.p.	n.p.	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant	recombinant
<i>Buffer for isolation of receptor</i>	n.a.	n.a.	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DDT, 10% glycerol containing 1 mg/mL BSA pH 7.6)
<i>Protein concentration</i>	1.2 nM	1.2 nM	n.p.
Competitive binding assay			
<i>Radioligand used</i>	^3H -17 -estradiol	^3H -17 -estradiol	^3H -17 -estradiol
<i>Concentration of radioligand</i>	2.5 nM	2.5 nM	10 pM - 1 μM
<i>Solvent used to dissolve ligand</i>	n.p.	n.p.	DMSO
<i>Concentration range of competing ligand</i>	5 nM - 100 μM	0.1 μM - 10 μM	60 nM - 20 μM
<i>Number of replicates</i>	3	3	4
<i>Number of times assay repeated</i>	3	2	3
<i>Time of incubation</i>	4 hours	4 hours	24 hours
<i>Temperature of incubation</i>	room temperature	room temperature	4°C
<i>Nonspecific binding measured (y/n)</i>	y	y	n.p.
Separation of ligand			
<i>Type of slurry</i>	hydroxyapatite	hydroxyapatite	n.p.
<i>Incubation time and temperature</i>	15 min; n.p.	15 min; n.p.	n.p.
<i>Centrifugation time and temperature</i>	20 min; n.p.	20 min; n.p.	n.p.
Data calculations			
<i>Program or method used for calculating data</i>	SigmaPlot	SigmaPlot	Nonlinear regression using Graphpad Prism 3.0
<i>Data plotted as</i>	% ^3H -E ₂ bound vs. log M of ligand	% ^3H -E ₂ bound vs. log M of ligand	Specific binding vs. log competitor conc.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	IC ₅₀	IC ₅₀
<i>Calculation of RBA</i>	n.p.	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor

Abbreviations: n.p. = not provided;
 n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ER α and ER β

Reference	Gaido et al. (1999)	Klotz et al. (1996)	Kraichely et al. (2000)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER alpha and human ER beta	human ER alpha	human ER alpha and human ER beta
<i>Source of receptor</i>	PanVera	Produced in Sf9 insect cells using a baculovirus expression system	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant	recombinant
<i>Buffer for isolation of receptor</i>	n.a.	n.p.	n.a.
<i>Protein concentration</i>	8 pmol/mL (alpha) 11 pmol/mL (beta)	0.4 nM	1.5 nM
Competitive binding assay			
<i>Radioligand used</i>	^3H -17 -estradiol	^3H -17 -estradiol	^3H -17 -estradiol
<i>Concentration of radioligand</i>	5 nM	2.5 nM	10 nM
<i>Solvent used to dissolve ligand</i>	n.p.	dimethyl sulfoxide or ethanol	n.p.
<i>Concentration range of competing ligand</i>	0.1 nM - 10 μM	10 nM - 100 μM	n.p.
<i>Number of replicates</i>	3	3	n.p.
<i>Number of times assay repeated</i>	3	2	n.p.
<i>Time of incubation</i>	overnight	1 hour	18 hours
<i>Temperature of incubation</i>	4°C	25°C	0°C
<i>Nonspecific binding measured (y/n)</i>	n.p.	y	n.p.
Separation of ligand			
<i>Type of slurry</i>	hydroxyapatite	5% activated charcoal/0.5% dextran	hydroxyapatite
<i>Incubation time and temperature</i>	30 min; 4°C	10 min; 4°C	15 min; 0°C
<i>Centrifugation time and temperature</i>	10 min; n.p.	3 min; n.p.	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer
Data calculations			
<i>Program or method used for calculating data</i>	GraphPad Prism software	n.p.	n.p.
<i>Data plotted as</i>	% Binding vs. log dose (M)	% ^3H -E ₂ bound vs. [ligand] in nM	no plot of data reported
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	RBA	IC ₅₀	RBA
<i>Calculation of RBA</i>	IC ₅₀ E ₂ /IC ₅₀ competitor	n.p.	n.p.

Abbreviations: n.p. = not provided;

n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ER α and ER β

Reference	Meyers et al. (1999)	Sun et al. (1999)	Sun et al. (1999)
Preparation of receptor			
<i>Species and subtype of receptor</i>	human ER alpha and human ER beta	human ER alpha ligand binding domain	human ER beta ligand binding domain
<i>Source of receptor</i>	PanVera	expressed in E coli using pET15b vector	expressed in E coli using pET15b vector
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	truncated (amino acids 304 - 554)	truncated (amino acids 256 - 505)
<i>Buffer for isolation of receptor</i>	n.a.	50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol	50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol
<i>Protein concentration</i>	1.5 nM	n.p.	n.p.
Competitive binding assay			
<i>Radioligand used</i>	^3H -17 -estradiol	^3H -17 -estradiol	^3H -17 -estradiol
<i>Concentration of radioligand</i>	10 nM	10 nM	10 nM
<i>Solvent used to dissolve ligand</i>	n.p.	n.p.	n.p.
<i>Concentration range of competing ligand</i>	n.p.	n.p.	n.p.
<i>Number of replicates</i>	2	n.p.	n.p.
<i>Number of times assay repeated</i>	n.p.	n.p.	n.p.
<i>Time of incubation</i>	18 - 24 hours	18 hours	18 hours
<i>Temperature of incubation</i>	0°C	0°C	0°C
<i>Nonspecific binding measured (y/n)</i>	n.p.	n.p.	n.p.
Separation of ligand			
<i>Type of slurry</i>	hydroxyapatite	hydroxylapatite	hydroxylapatite
<i>Incubation time and temperature</i>	15 min; 0°C	15 min; 0°C	15 min; 0°C
<i>Centrifugation time and temperature</i>	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer
Data calculations			
<i>Program or method used for calculating data</i>	n.p.	Ki calculated using Cheng-Prusoff equation	Ki calculated using Cheng-Prusoff equation
<i>Data plotted as</i>	no plot of data reported	no plot of data reported	no plot of data reported
<i>Data format in paper (e.g., IC₅₀, Ki)</i>	RBA	IC ₅₀ (not reported), Ki, and RBA	IC ₅₀ (not reported), Ki, and RBA
<i>Calculation of RBA</i>	n.p.	IC ₅₀ E ₂ /IC ₅₀ competitor x100	IC ₅₀ E ₂ /IC ₅₀ competitor x100

Abbreviations: n.p. = not provided;
 n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ER α and ER β

Reference	Vakharia and Gierthy (1999)	Vakharia and Gierthy (2000)
Preparation of receptor		
<i>Species and subtype of receptor</i>	human ER alpha	human ER alpha
<i>Source of receptor</i>	PanVera	PanVera
<i>Whole, truncated, recombinant, or chimeric</i>	recombinant	recombinant
<i>Buffer for isolation of receptor</i>	n.a.	n.a.
<i>Protein concentration</i>	1.2 nM	1.25 nM
Competitive binding assay		
<i>Radioligand used</i>	^3H -17 β -estradiol	^3H -17 β -estradiol
<i>Concentration of radioligand</i>	2.5 nM	2.5 nM
<i>Solvent used to dissolve ligand</i>	dimethyl sulfoxide	dimethyl sulfoxide
<i>Concentration range of competing ligand</i>	10 nM -1000 μM	50 nM - 50 μM
<i>Number of replicates</i>	3	3
<i>Number of times assay repeated</i>	n.p.	n.p.
<i>Time of incubation</i>	4 hours	4 hours
<i>Temperature of incubation</i>	room temperature	room temperature
<i>Nonspecific binding measured (y/n)</i>	y	y
Separation of ligand		
<i>Type of slurry</i>	hydroxyapatite	hydroxyapatite
<i>Incubation time and temperature</i>	n.p.	n.p.
<i>Centrifugation time and temperature</i>	10 min; n.p.	10 min; n.p.
Data calculations		
<i>Program or method used for calculating data</i>	Sigmplot software	Sigmplot software
<i>Data plotted as</i>	% ^3H -E ₂ bound vs. [ligand] in nM	% ^3H -E ₂ bound vs. [ligand] in nM
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	IC ₅₀
<i>Calculation of RBA</i>	n.a.	n.a.

Abbreviations: n.p. = not provided;
 n.a. = not applicable; RBA = relative binding affinity